

Untitled SEQUENCE LISTING

<110> Noteborn, Mathieu

Rohn, Jennifer Leigh

Mumberg, Dominik

Donner, Peter

<120> Modifications of Apoptin

<130> 2906-4996.1

<140> 10/083,849

<141> 2001-10-19

<150> US 60/242,397

<151> 2000-10-20

<160> 20

<170> PatentIn version 3.1

<210> 1

<211> 121

<212> PRT

<220>

<221> MISC FEATURE

<222> (1)..(121)

<223> Apoptin (a small protein derived from chicken anemia virus) enc od ed by pCMV-Vp3 and by GFP-Apoptin constructs

<400> 1

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70. 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

Page 2

<210> 2 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121)Apoptin protein encoded by pIRESneo alanine mutants <223> <220> <221> MISC FEATURE Differs from Apoptin protein encoded by pCMV-Vp3 and by GFP-Apo <223> pt in constructs by replacement of the arginine residue at positio n 116 with a lysine residue <400> 2

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Lys Arg Arg Ile Arg Leu 115 120

<210> 3

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-86 of 5-alanine linker-scanning mutant series of Ap optin

<400> 3

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Ala Ala Ala Ala Ala Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 4

<211> 121

<212> .PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-91 of 5-alanine linker-scanning mutant series of Ap optin

<400> 4

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Ala Ala Ala Ala Ala Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu. 115 120

<210> 5

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<400> 5.

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Ala 85 90 95

Ala Ala Ala Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 6

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-101 of 5-alanine linker-scanning mutant series of A poptin

<400> 6

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Ala Ala Ala Ala Ala Thr Thr Pro Ser Arg Pro-100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 7

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-106 of 5-alanine linker-scanning mutant series of A poptin

<400> 7

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Ala Ala Ala Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 8

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-111 of 5-alanine linker-scanning mutant series of A poptin

<400> 8

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Ala Ala 100 105 110

Ala Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 9

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-116 of 5-alanine linker-scanning mutant series of A poptin

<400> 9

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Ala Ala Ala Ala Leu 115 120

<210> 10

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106A of Apoptin

<400> 10

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 11

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107A of Apoptin

<400> 11

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 12

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108A of Apoptin

<400> 12

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 . 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 13

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant P109A of Apoptin

<400> 13

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Ala Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 14

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106E of Apoptin

<400> 14

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Page 17

Val Ser Glu Leu Lys Glu Ser Leu Ile Glu Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 15

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107E of Apoptin

<400> 15

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Glu Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 16

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108E of Apoptin

<400> 16

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Page 19

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Glu Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 17

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutation T106A107A of Apoptin

<400> 17

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Thr Pro Ser Arg Pro .100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 18

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T107A108A of Apoptin

<400> 18

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 19

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T106A108A of Apoptin

<400> 19

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 20

<211> 8

<212> PRT

<213> Chicken anemia virus

<221> MISC_FEATURE

<223> amino acid sequence encoding the SV40-Large T nuclear localizat io n signal

<400> 20

Pro Pro Lys Lys Lys Arg Lys Val